

~~10/501671~~

DT04 Rec'd PCT/PTO 16 JUL 2004

Sequence Listing

<110> Asahi Kasei Kabushiki Kaisha

<120> High-concentration preparation of soluble thrombomodulin

<130> ASAHI-33

<150> JP2002-009951

<151> 2002-01-18

<160> 9

<210> 1

<211> 516

<212> PRT

<213> Artificial sequence

<220>

<223> Partial amino acid sequence of human-originated soluble thrombomodulin

<400> 1

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly

1

5

10

15

Phe Pro Ala Pro Ala Glu Pro Gln Pro Gly Gly Ser Gln Cys Val Glu

20

25

30

His Asp Cys Phe Ala Leu Tyr Pro Gly Pro Ala Thr Phe Leu Asn Ala

35

40

45

Ser Gln Ile Cys Asp Gly Leu Arg Gly His Leu Met Thr Val Arg Ser

50	55	60
Ser Val Ala Ala Asp Val Ile Ser Leu Leu Leu Asn Gly Asp Gly Gly		
65	70	75
Val Gly Arg Arg Arg Leu Trp Ile Gly Leu Gln Leu Pro Pro Gly Cys		
85	90	95
Gly Asp Pro Lys Arg Leu Gly Pro Leu Arg Gly Phe Gln Trp Val Thr		
100	105	110
Gly Asp Asn Asn Thr Ser Tyr Ser Arg Trp Ala Arg Leu Asp Leu Asn		
115	120	125
Gly Ala Pro Leu Cys Gly Pro Leu Cys Val Ala Val Ser Ala Ala Glu		
130	135	140
Ala Thr Val Pro Ser Glu Pro Ile Trp Glu Glu Gln Gln Cys Glu Val		
145	150	155
Lys Ala Asp Gly Phe Leu Cys Glu Phe His Phe Pro Ala Thr Cys Arg		
165	170	175
Pro Leu Ala Val Glu Pro Gly Ala Ala Ala Ala Val Ser Ile Thr		
180	185	190
Tyr Gly Thr Pro Phe Ala Ala Arg Gly Ala Asp Phe Gln Ala Leu Pro		
195	200	205
Val Gly Ser Ser Ala Ala Val Ala Pro Leu Gly Leu Gln Leu Met Cys		
210	215	220
Thr Ala Pro Pro Gly Ala Val Gln Gly His Trp Ala Arg Glu Ala Pro		
225	230	235
Gly Ala Trp Asp Cys Ser Val Glu Asn Gly Gly Cys Glu His Ala Cys		
245	250	255
Asn Ala Ile Pro Gly Ala Pro Arg Cys Gln Cys Pro Ala Gly Ala Ala		
260	265	270
Leu Gln Ala Asp Gly Arg Ser Cys Thr Ala Ser Ala Thr Gln Ser Cys		
275	280	285

Asn Asp Leu Cys Glu His Phe Cys Val Pro Asn Pro Gln Pro Gly  
290 295 300  
Ser Tyr Ser Cys Met Cys Glu Thr Gly Tyr Arg Leu Ala Ala Asp Gln  
305 310 315 320  
His Arg Cys Glu Asp Val Asp Asp Cys Ile Leu Glu Pro Ser Pro Cys  
325 330 335  
Pro Gln Arg Cys Val Asn Thr Gln Gly Gly Phe Glu Cys His Cys Tyr  
340 345 350  
Pro Asn Tyr Asp Leu Val Asp Gly Glu Cys Val Glu Pro Val Asp Pro  
355 360 365  
Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro Leu Asn Gln Thr  
370 375 380  
Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro Ile Pro His Glu  
385 390 395 400  
Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala Cys Pro Ala Asp  
405 410 415  
Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro Glu Gly Tyr Ile  
420 425 430  
Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu Cys Glu Asn Gly  
435 440 445  
Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly Thr Phe Glu Cys  
450 455 460  
Ile Cys Gly Pro Asp Ser Ala Leu Val Arg His Ile Gly Thr Asp Cys  
465 470 475 480  
Asp Ser Gly Lys Val Asp Gly Gly Asp Ser Gly Ser Gly Glu Pro Pro  
485 490 495  
Pro Ser Pro Thr Pro Gly Ser Thr Leu Thr Pro Pro Ala Val Gly Leu  
500 505 510  
Val His Ser Gly

<210> 2  
<211> 1548  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Partial base sequence of human-originated soluble  
thrombomodulin gene

<400> 2

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gcagagccgc agccgggtgg cagccagtgc gtcgagcacf actgcttcgc gctctacccg 120  
ggccccgcga ctttcctcaa tgccagtcag atctgcgacg gactgcgggg ccacctaattg 180  
acagtgcgct cctcgggtggc tgccgatgtc atttccttgc tactgaacgg cgacggccgc 240  
gttggccgcc ggcgcctctg gatcggcctg cagctgccac ccggctgcgg cgaccccaag 300  
cgccctcgccc ccctgcgcgg cttccagtgg gttacgggag acaacaacac cagctatacg 360  
agggtggcac ggctcgaccc caatggggct cccctctgcg gcccgttgtg cgtcgctgtc 420  
tccgctgctg aggccactgt gcccagcgcg ccgatctggg aggagcagca gtgcgaagtg 480  
aaggccgatg gcttcctctg cgagttccac ttcccagcca cctgcaggcc actggctgtg 540  
gagccccggcg ccgcggctgc cgccgtctcg atcacctacg gcaccccggtt cgccggccgc 600  
ggagcggact tccaggcgct gccgggtggc agctccgcgg cggtggctcc cctcggctta 660  
cagctaattgt gcaccgcgcg gcccggagcg gtccaggggc actggccag ggaggcgccg 720  
ggcgcttggg actgcagcgt ggagaacggc ggctgcgagc acgcgtgcaa tgcgatccct 780  
ggggctcccc gctgccagtg cccagccggc gcccgcctgc aggcagacgg gcgctcctgc 840  
accgcatccg cgacgcagtc ctgcaacgc acgtgcgagc acttctgcgt tcccaacccc 900  
gaccagccgg gctcctactc gtgcattgtgc gagaccggct accggctggc ggccgaccaa 960  
caccgggtgcg aggacgtgga tgactgcata ctggagccca gtccgtgtcc gcagcgctgt 1020

gtcaacacac agggtggctt cgagtgccac tgctacccta actacgacct ggtggacggc 1080  
gagtgtgtgg agcccgtgga cccgtgcttc agagccaact gcgagtagcca gtgccagccc 1140  
ctgaaccaaa ctagctaccc ctgcgtctgc gccgagggtc tcgcgcctat tccccacgag 1200  
ccgcacaggt gccagatgtt ttgcaaccag actgcctgtc cagccgactg cgaccccaac 1260  
acccaggcta gctgtgagtg ccctgaaggc tacatcctgg acgacggttt catctgcacg 1320  
gacatcgacg agtgcgaaaa cggcggttc tgctccgggg tgtgccacaa cctccccgt 1380  
accttcgagt gcatctgcgg gcccgactcg gcccttgc gcccacattgg caccgac. t 1440  
gactccggca aggtggacgg tggcgacagc ggctctggcg agccccggc cagccccgacg 1500  
cccggttcca ctttgactcc tccggccgtg gggctcgtgc attcgggc 1548

<210> 3

<211> 132

<212> PRT

<213> Artificial sequence

<220>

<223> Partial amino acid sequence of human-originated soluble  
thrombomodulin

<400> 8

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly

1 5 10 15

Phe Pro Asp Pro Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro

20 25 30

Leu Asn Gln Thr Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro

35 40 45

Ile Pro His Glu Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala

50 55 60

Cys Pro Ala Asp Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro

65                   70                   75                   80  
Glu Gly Tyr Ile Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu  
85                   90                   95  
Cys Glu Asn Gly Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly  
100                  105                  110  
Thr Phe Glu Cys Ile Cys Gly Pro Asp Ser Ala Leu Val Arg His Ile  
115                  120                  125  
Gly Thr Asp Cys  
130

<210> 4

<211> 396

<212> DNA

<213> Artificial sequence

<220>

<223> Partial base sequence of human-originated soluble  
thrombomodulin gene

<400> 4

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tgcttcagag ccaactgcga gtaccagtgc cagcccctga accaaactag ctacacctgc 120  
gtctgcgccg agggcttcgc gcccattccc cacgagccgc acaggtgcc aatgtttgc 180  
aaccagactg cctgtccagc cgactgcgac cccaacaccc aggcttagctg tgagtgcct 240  
gaaggctaca tcctggacga cggttcatc tgcacggaca tcgacgagtg cgaaaacggc 300  
ggcttctgct ccgggggtgtg ccacaacctc cccggtacct tcgagtgcat ctgcggggcc 360  
gactcggccc ttgtccgcca cattggcacc gactgt                           396

<210> 5

<211> 516

<212> PRT

<213> Artificial sequence

<220>

<223> Partial amino acid sequence of human-originated soluble  
thrombomodulin

<400> 5

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly

1 5 10 15

Phe Pro Ala Pro Ala Glu Pro Gln Pro Gly Gly Ser Gln Cys Val Glu

20 25 30

His Asp Cys Phe Ala Leu Tyr Pro Gly Pro Ala Thr Phe Leu Asn Ala

35 40 45

Ser Gln Ile Cys Asp Gly Leu Arg Gly His Leu Met Thr Val Arg Ser

50 55 60

Ser Val Ala Ala Asp Val Ile Ser Leu Leu Leu Asn Gly Asp Gly Gly

65 70 75 80

Val Gly Arg Arg Arg Leu Trp Ile Gly Leu Gln Leu Pro Pro Gly Cys

85 90 95

Gly Asp Pro Lys Arg Leu Gly Pro Leu Arg Gly Phe Gln Trp Val Thr

100 105 110

Gly Asp Asn Asn Thr Ser Tyr Ser Arg Trp Ala Arg Leu Asp Leu Asn

115 120 125

Gly Ala Pro Leu Cys Gly Pro Leu Cys Val Ala Val Ser Ala Ala Glu

130 135 140

Ala Thr Val Pro Ser Glu Pro Ile Trp Glu Glu Gln Gln Cys Glu Val

145 150 155 160

Lys Ala Asp Gly Phe Leu Cys Glu Phe His Pro Ala Thr Cys Arg  
                  165                 170                 175  
 Pro Leu Ala Val Glu Pro Gly Ala Ala Ala Ala Val Ser Ile Thr  
                  180                 185                 190  
 Tyr Gly Thr Pro Phe Ala Ala Arg Gly Ala Asp Phe Gln Ala Leu Pro  
                  195                 200                 205  
 Val Gly Ser Ser Ala Ala Val Ala Pro Leu Gly Leu Gln Leu Met Cys  
                  210                 215                 220  
 Thr Ala Pro Pro Gly Ala Val Gln Gly His Trp Ala Arg Glu Ala Pro  
                  225                 230                 235                 240  
 Gly Ala Trp Asp Cys Ser Val Glu Asn Gly Gly Cys Glu His Ala Cys  
                  245                 250                 255  
 Asn Ala Ile Pro Gly Ala Pro Arg Cys Gln Cys Pro Ala Gly Ala Ala  
                  260                 265                 270  
 Leu Gln Ala Asp Gly Arg Ser Cys Thr Ala Ser Ala Thr Gln Ser Cys  
                  275                 280                 285  
 Asn Asp Leu Cys Glu His Phe Cys Val Pro Asn Pro Asp Gln Pro Gly  
                  290                 295                 300  
 Ser Tyr Ser Cys Met Cys Glu Thr Gly Tyr Arg Leu Ala Ala Asp Gln  
                  305                 310                 315                 320  
 His Arg Cys Glu Asp Val Asp Asp Cys Ile Leu Glu Pro Ser Pro Cys  
                  325                 330                 335  
 Pro Gln Arg Cys Val Asn Thr Gln Gly Gly Phe Glu Cys His Cys Tyr  
                  340                 345                 350  
 Pro Asn Tyr Asp Leu Val Asp Gly Glu Cys Val Glu Pro Val Asp Pro  
                  355                 360                 365  
 Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro Leu Asn Gln Thr  
                  370                 375                 380  
 Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro Ile Pro His Glu

385                   390                   395                   400  
Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala Cys Pro Ala Asp  
405                   410                   415  
Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro Glu Gly Tyr Ile  
420                   425                   430  
Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu Cys Glu Asn Gly  
435                   440                   445  
Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly Thr Phe Glu Cys  
450                   455                   460  
Ile Cys Gly Pro Asp Ser Ala Leu Ala Arg His Ile Gly Thr Asp Cys  
465                   470                   475                   480  
Asp Ser Gly Lys Val Asp Gly Gly Asp Ser Gly Ser Gly Glu Pro Pro  
485                   490                   495  
Pro Ser Pro Thr Pro Gly Ser Thr Leu Thr Pro Pro Ala Val Gly Leu  
500                   505                   510  
Val His Ser Gly  
515

<210> 6  
<211> 1548  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Partial base sequence of human-originated soluble  
thrombomodulin gene

<400> 6  
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ggccccgcga cttcctcaa tgccagtca gatctgcgacg gactgcgggg ccacctaatt 180  
acagtgcgct cctcggtggc tgccgatgtc atttccttgc tactgaacgg cgacggccgc 240  
gttggccgcc ggccgcctctg gatcggcctg cagctgccac ccggctgcgg cgaccccaag 300  
cgccctcgccc ccctgcgcgg cttccagtgg gttacgggag acaacaacac cagctatacg 360  
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aaggccgatg gcttcctctg cgagttccac ttcccagcca cctgcaggcc actggctgtc 540  
gagcccggcg ccgcggctgc cgccgtctcg atcacctacg gcaccccggtt cgccggccgc 600  
ggagcggact tccaggcgct gccgggtggc agctccgccc cggtggtcc cctcggctta 660  
cagctaattgt gcaccgcgcgccc gcccggagcg gtccaggggc actggccag ggaggcgccg 720  
ggcgcttggg actgcagcggt ggagaacggc ggctgcgagc acgcgtgcaa tgcgatccct 780  
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accgcattccg cgacgcagtc ctgcaacgc acctgcgagc acttctgcgt tcccaacccc 900  
gaccagccgg gctcctactc gtgcattgtc gagaccggct accggctggc ggccgaccaa 960  
caccggtgcg aggacgtgga tgactgcata ctggagccca gtccgtgtcc gcagcgctgt 1020  
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gactccggca aggtggacgg tggcgacagc ggctctggcg agccccggcc cagccccgacg 1500  
cccggttcca ctttgactcc tccggccgtg gggctcgtgc attcgggc 1548

<210> 7

<211> 132

<212> PRT

<213> Artificial sequence

<220>

<223> Partial amino acid sequence of human-originated soluble thrombomodulin

<400> 7

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly

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Phe Pro Asp Pro Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro

20 . . . . . 25 . . . . . 30 . . . . .

Leu Asn Gln Thr Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro

35 . . . . . 40 . . . . . 45 . . . . .

Ile Pro His Glu Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala

50 . . . . . 55 . . . . . 60 . . . . .

Cys Pro Ala Asp Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro

65 . . . . . 70 . . . . . 75 . . . . . 80 . . . . .

Glu Gly Tyr Ile Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu

85 . . . . . 90 . . . . . 95 . . . . .

Cys Glu Asn Gly Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly

100 . . . . . 105 . . . . . 110 . . . . .

Thr Phe Glu Cys Ile Cys Gly Pro Asp Ser Ala Leu Ala Arg His Ile

115 . . . . . 120 . . . . . 125 . . . . .

Gly Thr Asp Cys

130

<210> 8

<211> 396

<212> DNA

<213> Artificial sequence

<220>

<223> Partial base sequence of human-originated soluble  
thrombomodulin gene

<400> 8

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tgcttcagag ccaactgcga gtaccagtgc cagccctga accaaactag ctacctctgc 120  
gtctgcgccg agggcttcgc gcccattccc cacgagccgc acaggtgcca gatgtttgc 180  
aaccagactg cctgtccagc cgactgcgac cccaaacaccc aggctagctg tgagtgcct 240  
gaaggctaca tcctggacga cggttcatc tgcacggaca tcgacgagtg cgaaaacggc 300  
ggcttctgct ccggggtgtg ccacaacctc cccggtacct tcgagtgcac ctgcggggcc 360  
gactcggccc ttgcccccca cattggcacc gactgt 396

<210> 9

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic DNA for mutation

<400> 9

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21